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Sex Chromosomes: Evolution of the Weird and Wonderful

New findings in the platypus and *Drosophila pseudoobscura* illustrate, yet again, that the sex chromosomes seem never to stop evolving. Degeneration processes lead to a continual loss of genes and gene activity on the Y chromosome, and complete loss of Y-linked genes is possible if autosomal genes take over control of male fertility — though addition of new material to the sex chromosomes may start the process anew.

Deborah Charlesworth and Brian Charlesworth

I like the duck-billed platypus
Because it is anomalous.
I like the way it raises its family
Partly birdly, partly mammaly.
I like its independent attitude.
Let no one call it a duck-billed platitude.
Ogden Nash

Advanced sex chromosomes — XX/XY with male heterogamety, ZZ/ZW with female heterogamety — have evolved independently in many different lineages, and show several striking common features, including lack of recombination in the heterogametic sex, and genetic degeneration of the Y or W chromosome [1,2]. Degeneration involves the loss or inactivation of most Y- or W-linked genes, and this is often compensated for by sex differences in the expression of X or Z-linked genes [3]. The sex chromosomes are frequently heteromorphic, with the Y (W) often smaller than the X (Z), and largely made up of heterochromatin. Partially evolved sex chromosomes with only some of these features are also known [1]: in these cases, the non-recombining region of the chromosome pair that carries the sex determining genes forms only part of the chromosome, as in papaya [4] and the three-spined stickleback [5].

The initial reason for evolving lack of recombination between Y and X (or Z and W) chromosomes is probably because they originally carried sex-determining genes, and recombination between these genes would have produced disadvantageous sexual phenotypes [2]. It is thus not surprising that small non-recombining regions exist: these are presumably the regions where the sex-determining genes are located. But why do many sex chromosome systems have much larger non-recombining regions?

Such systems might have been created by chromosome inversions, which when heterozygous suppress crossing over across large regions, potentially including many genes other than those involved in sex determination, and contributing to the evolution of chromosome heteromorphism. The evolutionary pressure for this wider reduction of recombination is thought to come from the existence of loci with alleles that are sexually antagonistic — one allele is beneficial to males but harmful to females, and the other has the opposite effect. There is an obvious selective advantage to ensuring that such loci are closely linked to the sex-determining region [1,6,7].

These ideas apply to genes that are already on the sex-determining chromosome; linkage between sexually antagonistic loci on an autosome and the sex-determining region requires translocations bringing material from one chromosome onto another [8]. Translocations between the sex chromosomes and autosomes, creating 'neo-sex' chromosomes, have indeed become established in a variety of taxa, most often involving Robertsonian fusions between sex chromosomes and an autosome. For example, the *Drosophila pseudoobscura* X is made up of two arms, one homologous with the *D. melanogaster* 3L chromosome arm and one with its X (Figure 1). The eutherian mammalian Y is also a composite, made up of a part that was already X-linked in marsupials, and a part that became X-linked more recently, and which is still autosomal in marsupials [9]. An X-autosome fusion followed by a Y-autosome fusion (or vice versa) must have been involved in this case, as some genes in both parts are now present on the X as well as the Y. The DNA sequences of those still present in the added part have much lower X–Y divergence than the others, consistent with their alleles having stopped recombining more recently.

It now appears that the duck-billed platypus, a monotreme, is an extreme example of multiple

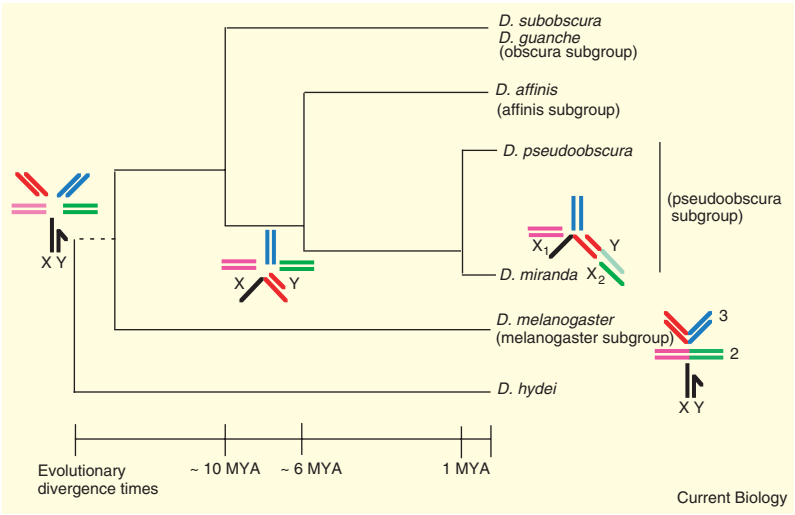


Figure 1. Chromosome arrangements in several *Drosophila* species, showing the fusions mentioned in the text and rough estimates of the times when they happened.

The male karyotypes are shown by the branches of the tree, and changed states are shown by the species in which they are found. The time estimates are based on synonymous site divergence values (K_s) for multiple genes between pairs of species, and are rough because there is no reliable molecular clock for *Drosophila* species. The approximate divergence values from *D. pseudoobscura* are: *D. miranda* 3.2%, *D. affinis* 23%, *D. melanogaster* 30% [20]. The divergence from either *D. melanogaster* or *D hydei* is so large that accurate dating is not possible (the dashed line indicates a long time). The *D. miranda* neo-Y is shown as a broken line to indicate that it is partially degenerate.

reciprocal translocations involving sex chromosomes and autosomes, with permanent translocation heterozygosity in males (which had previously been known to carry five chromosomes absent from females, out of a chromosome complement of 52 pairs). To sort out their homologies, chromosomes from a male were separated according to their sizes, and DNA fractions enriched for each particular chromosome were labelled to make 'chromosome paints' which can be used to identify the different chromosomes [10]. Five chromosome pairs form a chain in meiosis, and the paints establish that they share homologous arms, indicating that they were formed by reciprocal translocations between different chromosomes (Figure 2).

One member of the chain is homologous to the ancestral X of mammals, and the chromosomes that segregate from one another correspond to pairs of X- and Y-like chromosomes, the Y-like ones being seen only in males [10,11]. Another member of the chain — at the opposite end of the chain to the 'X' chromosome — carries the *DMRT1* gene, located on chromosome 9 in humans; *DMRT1* mutations cause sex reversal of males. *DMRT1* is carried on the Z chromosome of birds, and has been proposed as the bird female determining factor. Grützner *et al.* [11] suggest that the original platypus sex chromosome pair involved the one carrying *DMRT1*, not the homologue of the mammalian sex chromosomes. But the evidence for a role of *DMRT1* as primary sex determiner in birds is weak [12], so this finding could simply be coincidental, rather than implying a 'partly birdly, partly mammaly' sex-determination system for the platypus.

Although these findings seem odd, the platypus is far from uniquely anomalous [13], and even more extreme examples exist. Other well-studied cases are a termite species with a set of eight sex chromosome pairs, out of a chromosome complement of only 16 pairs [14]; some East African mistletoes have nine sex chromosomes, presumably corresponding to five pairs plus one too small to be seen (just like the platypus), out of a chromosome complement of 12 pairs plus one unpaired chromosome [15].

When another chromosome attaches to the sex chromosome, the resultant neo-Y may start degenerating, like other Y chromosomes, unless it continues to recombine with its homologue [1,2]. In the platypus, the neo-Ys could presumably recombine over part of their length, at least initially, so they might not degenerate completely. As with other examples of neo-sex chromosomes, it is not known what drove the successive exchanges of chromosome arms, but it seems unlikely that such similar events would have repeatedly happened so many times unless some advantage, such as sexual antagonism, was involved.

The neo-sex chromosomes of *D. pseudoobscura* and its relatives have also undergone further evolution, but differently from the platypus. At least two more transmutations have occurred. In its close relative *D. miranda*, a new translocation has joined the autosome homologous with the *D. melanogaster* 2R to the Y (Figure 1). The new arm is now partially degenerate, after only about one million years in a non-recombining state [16,17]. It now also seems that the ancestral Y may have degenerated completely in an ancestor of *D. pseudoobscura* and its relatives as distant as *D. affinis*, and that these species' existing Y may be a remnant of the Y_2 created by the earlier fusion, rather than the original Y chromosome as was formerly assumed [18].

The recent availability of sequences covering most of the *D. pseudoobscura* genome allowed searches for known *D. melanogaster* Y-linked genes; these are, as expected, present in *D. pseudoobscura*, but use of the polymerase chain reaction (PCR) with primers based on these sequences unexpectedly yielded products in both sexes, so they are certainly not sex-linked. Candidate *D. pseudoobscura* Y-linked genes

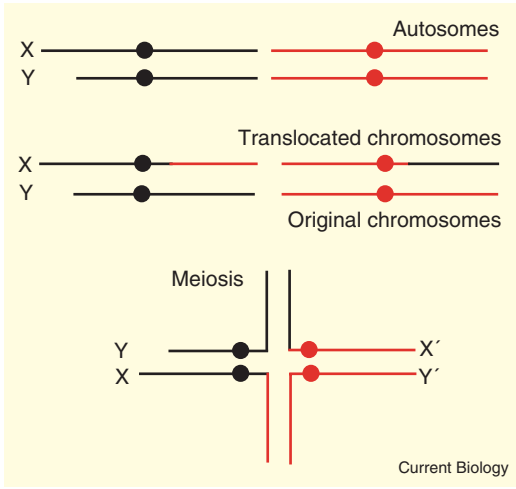
are also detectable (they have low representation in the genome sequence, and PCR tests detected them only in males). These are predominantly homologs of *D. melanogaster* 3L genes — 10 of 15 putatively Y-linked genes tested — and none is Y-linked in *D. melanogaster*.

These genes thus probably represent the degenerated remains of the neo-Y (Y_2). The ancestral Y must therefore have degenerated completely, presumably after the divergence, about 10-15 million years ago, of *D. pseudoobscura* from its relatives in the obscura subgroup, which lack the fusion (two species from the obscura subgroup were tested, and, as expected, they have the same Y-linked genes as *D. melanogaster*). Male higher Diptera have a meiosis without crossing-over, so *Drosophila* neo-Y chromosomes stop recombining as soon as they arise, which probably accounts for the small number of Y-linked genes remaining in *D. pseudoobscura*, the neo-Y of which is much older than that of *D. miranda*.

The *D. pseudoobscura* Y-linked genes also exist as non-Y-linked copies. Unfortunately, it is not yet known which chromosome they are on, and X-linkage was not tested for, but one would expect that they will probably all be found on the new X arm. No *D. melanogaster* Y-linked gene has a homologue on the X; all seem to have transposed onto the Y from autosomes [18], perhaps because their expression benefits males but harms females. But how then could these genes have been lost again from the *D. pseudoobscura* Y? There is good evidence that the Y in the *D. pseudoobscura* lineage must indeed have lost male fertility genes, as males of *D. pseudoobscura*'s close relative *D. affinis* lack a Y chromosome [19]. Presumably, a complex translocation event brought these genes onto an autosome [2], implying that these temporarily Y-linked genes should now be in a block on one *D. pseudoobscura* autosome, as seems to be the case from the contiguity of these genes in the *D. pseudoobscura* genome sequence [18].

Figure 2.

A reciprocal translocation between a sex chromosome pair and an autosome pair, showing the exchanged arms in the case of an X-autosome translocation (a Y-autosome translocation would produce similar results) when the Y chromosome differs morphologically from the X. In male meiosis, the segregation must ensure that each gamete receives either a Y chromosome plus a non-translocated autosome — which thus behaves like a Y chromosome and is passed from fathers to sons (Y′) — or else the pair of translocated chromosomes — X and X′, together carrying a complete complement of X-linked and autosomal loci.



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